Fig. 1A.

BamH I   Xba I

EcoRI   EcoR I

Ptac   Sph I

luc

bla

pPW601a
A215L/E354K

Stul

Pvul

Sall

gal K   EcoRI

Site directed
mutagenesis
BamHI → Nde I
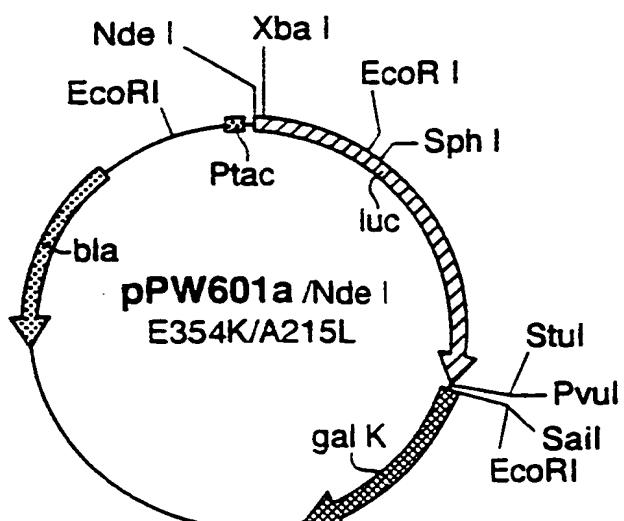
Nde I   Xba I
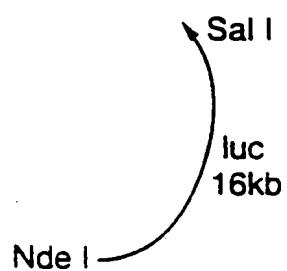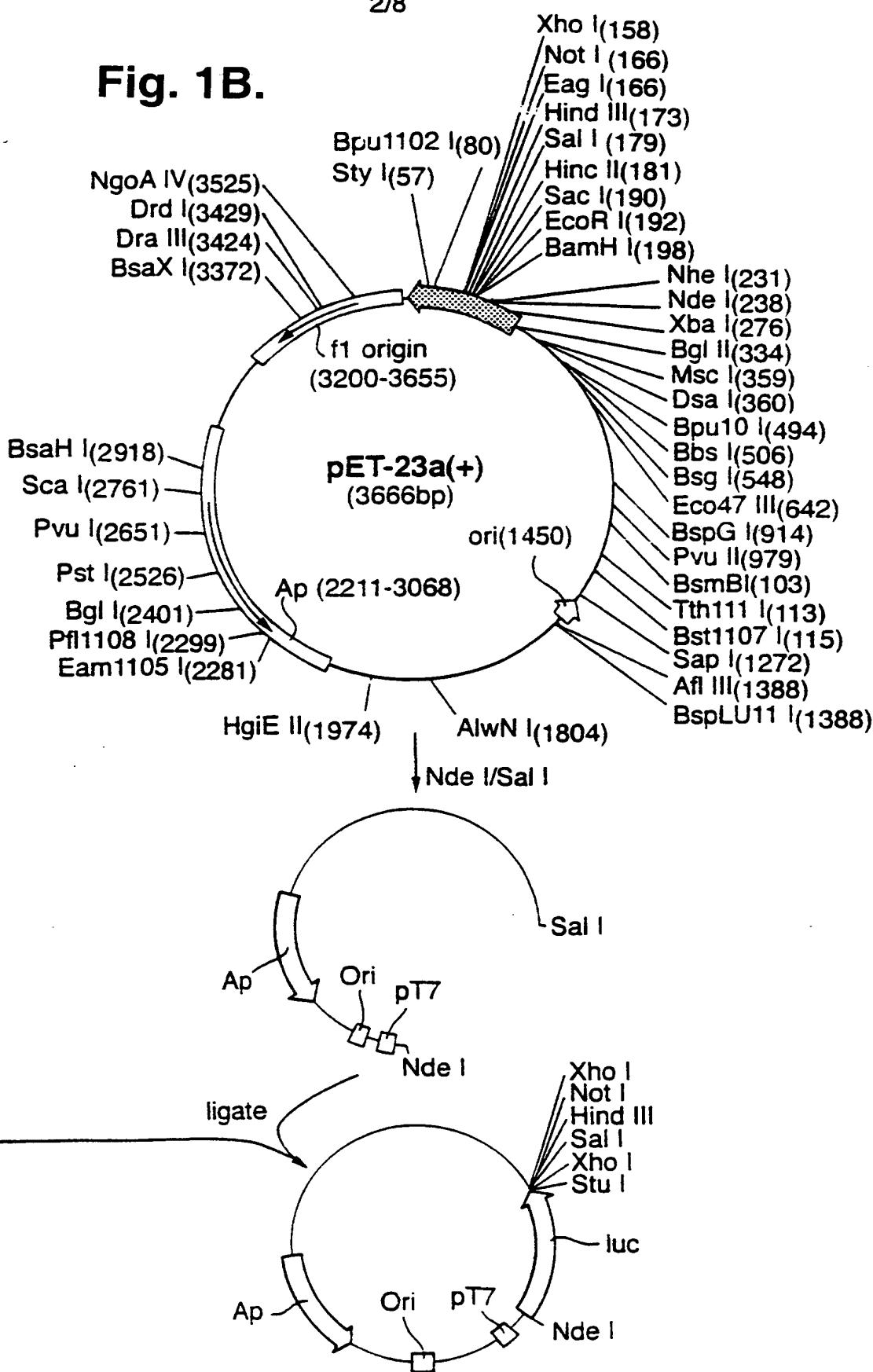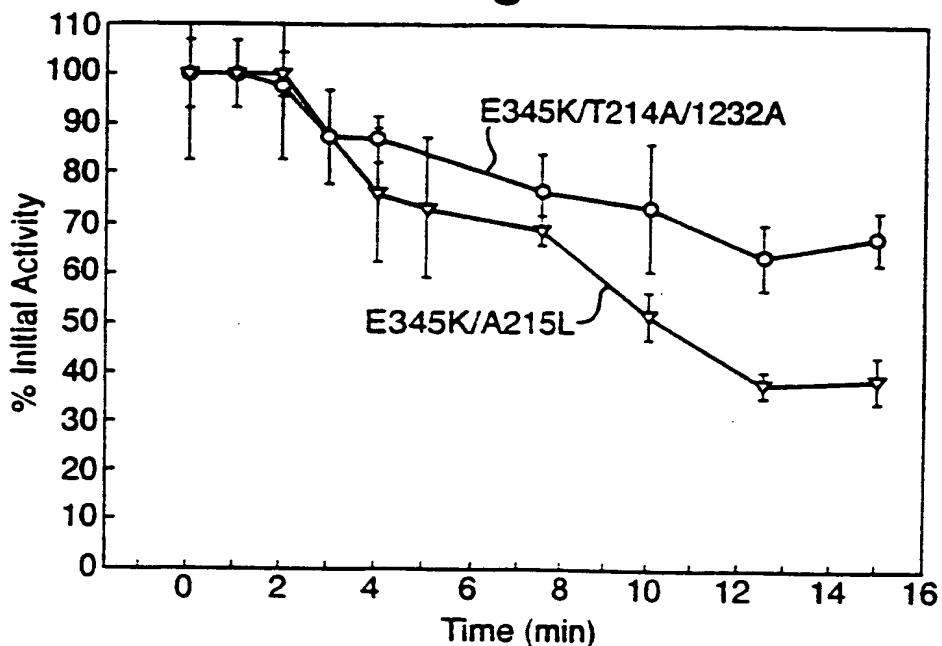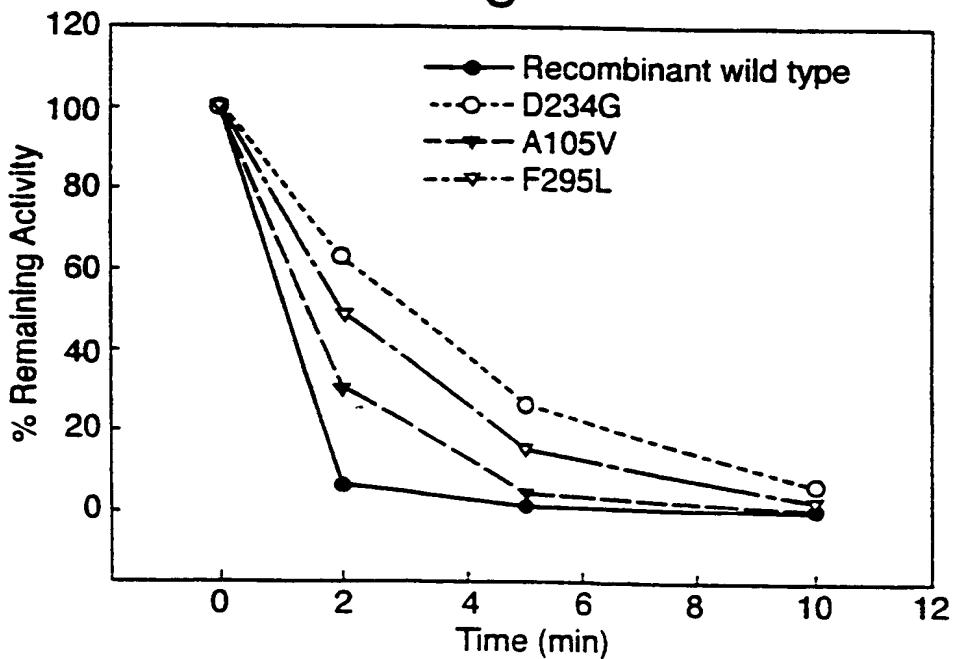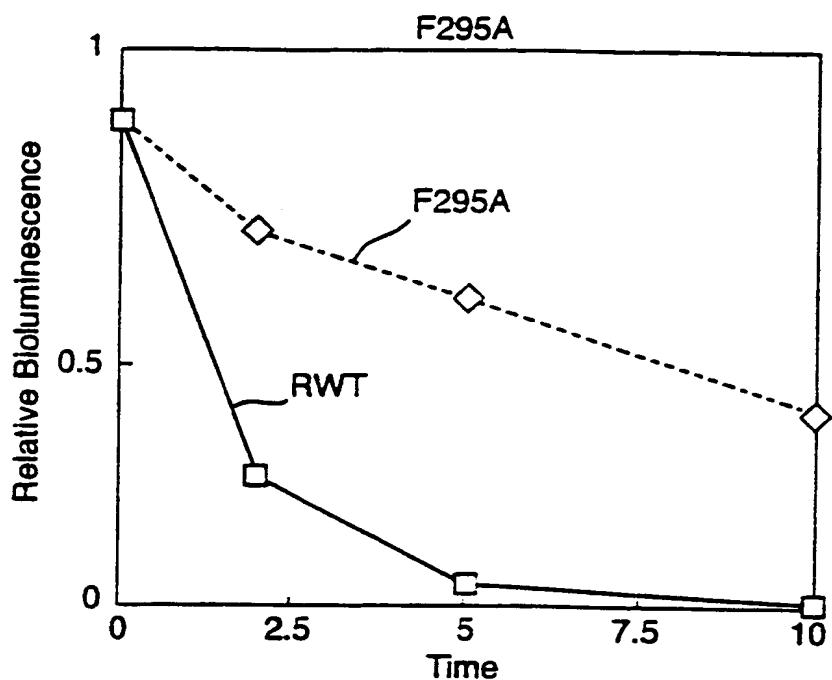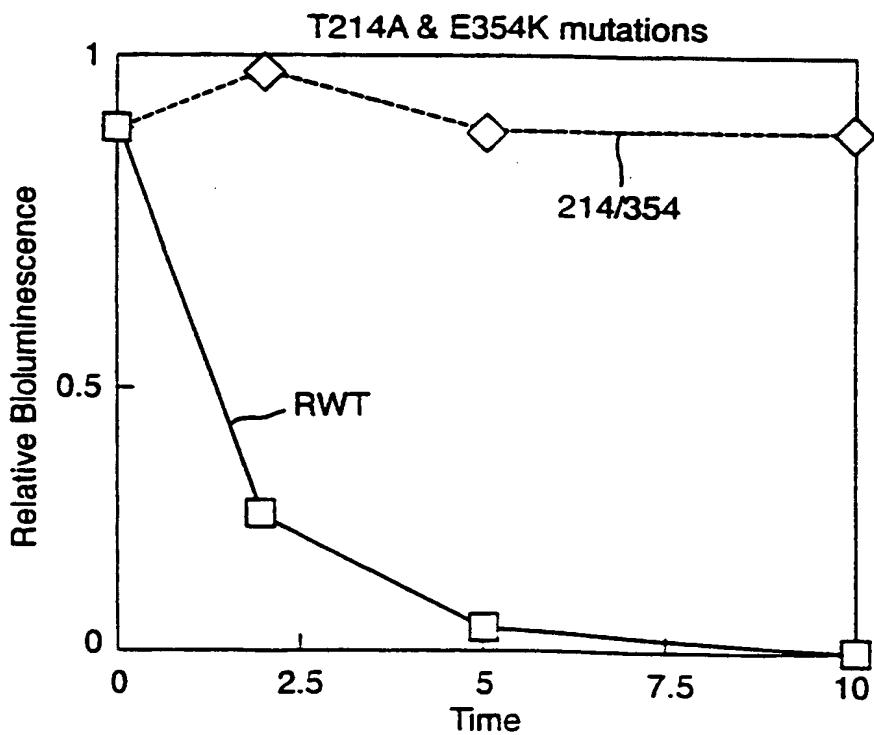
EcoRI   EcoR I

Ptac   Sph I

luc

bla

pPW601a /Nde I
E354K/A215L

Stul

Pvul

Sail

gal K   EcoRI

Nde I/Sal I

Sal I

luc
16kb

Nde I

Fig. 1B.

Fig.2.

E345K/T214A/1232A

E345K/A215L

% Initial Activity

Time (min)

Fig.4.

- ●— Recombinant wild type
- --○-- D234G
- --▼-- A105V
- --▽-- F295L

% Remaining Activity

Time (min)

Fig. 3A.

F295A

Fig. 3B.

T214A & E354K mutations

Fig. 3C.

T214A,I232A & E354K mutations

Fig. 3D.

T214A,T215L,I232A & E354K mutation

Fig. 3E.

1232A mutation

Fig. 3F.

T214A & E354K mutations (44°C)

Fig. 3G.

T214A,I232A & E354K mutations (44°C)

Fig. 3H.

T214A,A215L,I232A&E354K mutations (44°C)
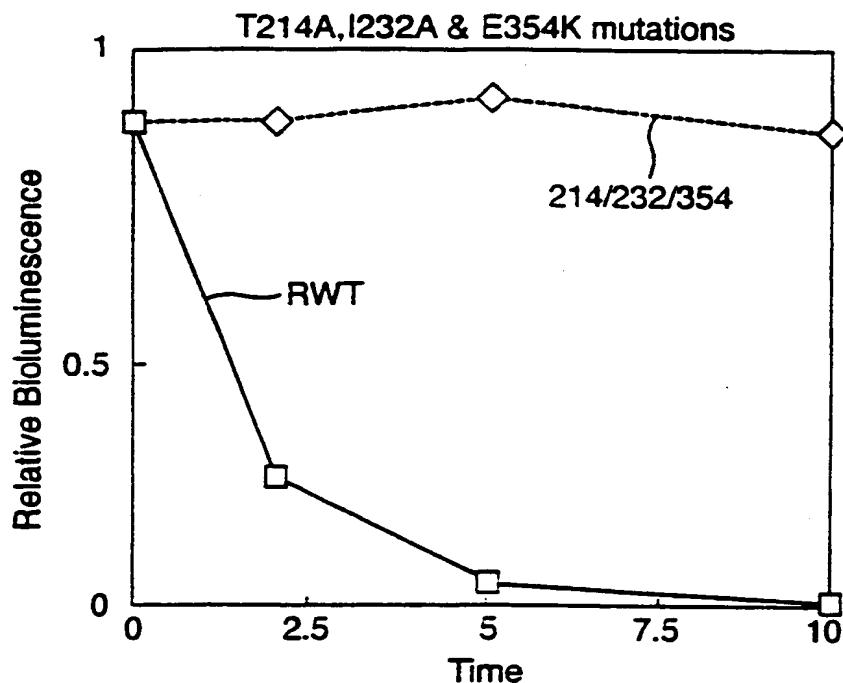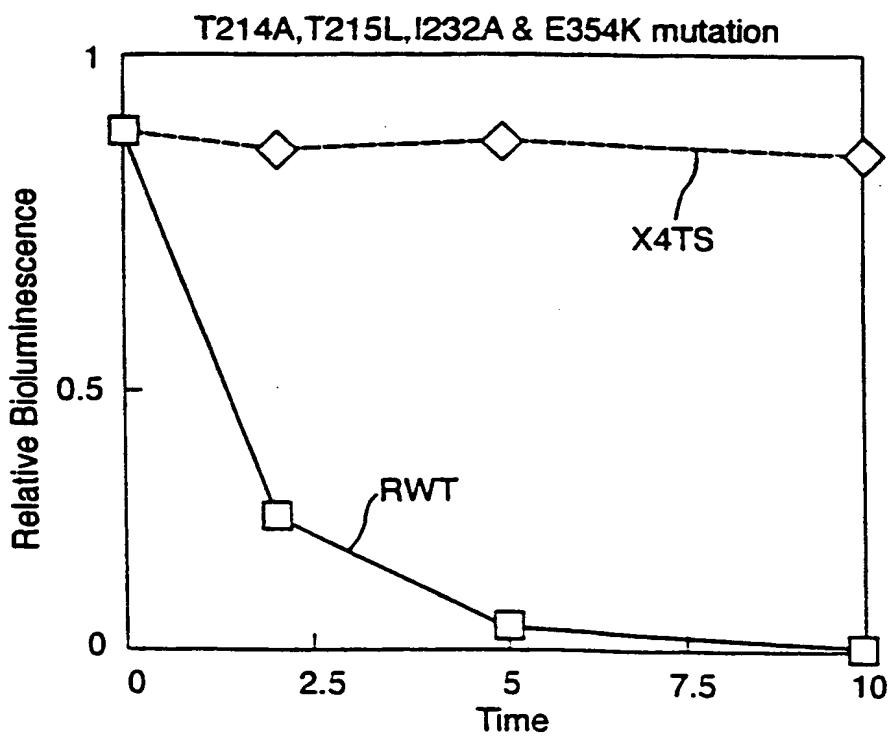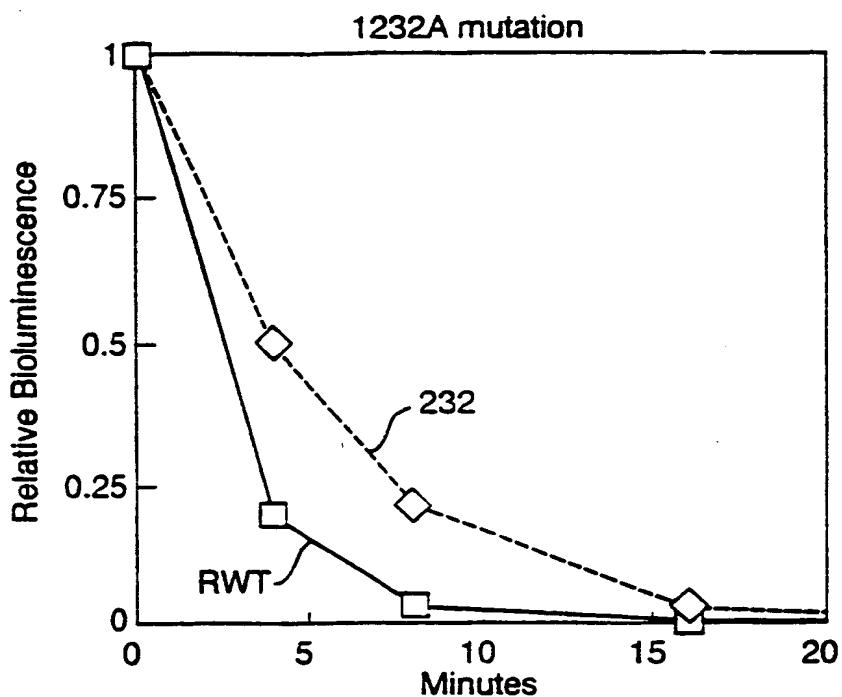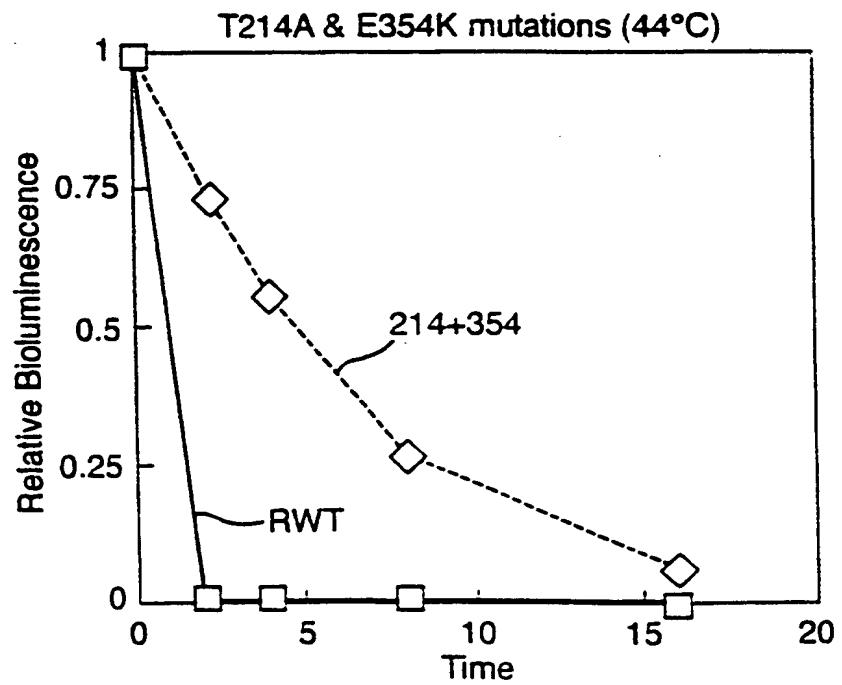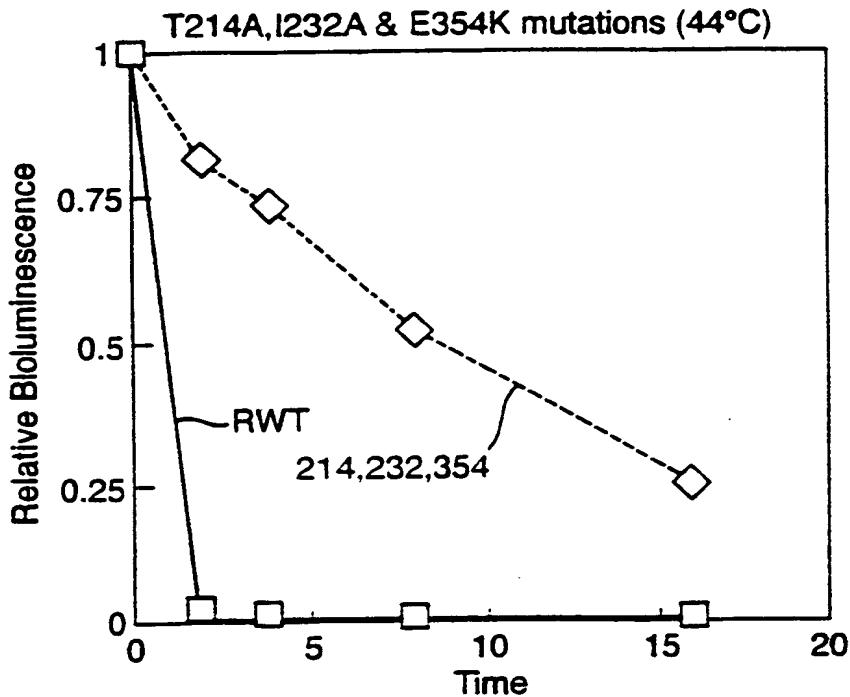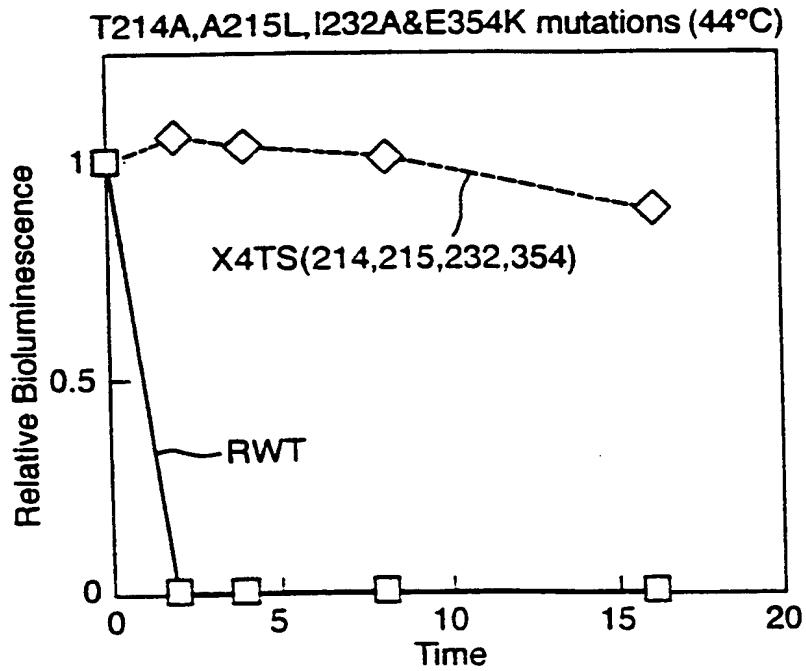
Fig. 5.

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CGCCGGTGAGCTCCCCGCCGCCG        SACI-SENSE / 6371
CGGCGGCGGGGAGCTCACCGGCG        SACI-ANTI / 6372
CGAACACTTCTTCATCGTTGACCGCCTTAAGTCTTTAATTAAATACAAAGG        AFLII-SENSE / 6373
CCTTTGTATTTAATTAAAGACTTAAGGCGGTCAACTATGAAGAAGTGTTCG        AFLII-ANTI / 6374
GAAAGGCCCGGCACCAGCCTATCCTCTAGAGG        F14A-SENSE / 6375
CCTCTAGCGGATAGGCTGGTGCCGGGCCTTTC        F14A-ANTI / 6376
CCATAAATTTACCGAATTCGTCGACTTCGATCGAGG        C-TERM.SEQ/ 6641
GTGTGGAATTGTGAGCGG        N-TERM.SEQ / 6651
GAGATACGCCGCGGTTCCTGG        L35A-SENSE / 6652
CCAGGAACCGCGGCGTATCTC        L35A-SENSE / 6653
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CCCTATTTTCATTCCTGGCCAAAAGCACTG                  F295L-SENSE/ 9048
GAGTGCTTTTGGCCAGGAATGAAAATAGGG                  F295L-ANTI / 9049
CCGCATAGAgCTCTCTGCGTCAGATTC              T214A + A215L-SENSE / 9063
GAATCTGACGCAGAGAGcTCTATGCGG              T214A + A215L-ANTI / 9064
GTTGACCGCTTGGGATCCTTAATTAAATAC           Insertion of BamHI at G339 / 9077
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GTATAGATTTGAAAAAGAGCTG             E270K-SENSE / 257
CAGCTCTTTTTCAAATCTATAC             E270K-ANTI / 258
GGCTACATACTGGAGACATAGC             S420T-SENSE / 629
GCTATGTCTCCAGTATGTAGCC             S420T-ANTI / 630
GCAGTTGCGCCCGTGAACGAC        A105L-SENSE / 790
GTCGTTCACGGGCGCAACTGC        A105L-ANTI /791
CAAATCATTCCGGGTACTGCGATTTTAAG            D234G-SENSE / 792
CTTAAAATCGCAGTACCCGGAATGATTTG            D234G-ANTI / 793
```

```
CCGCATAGAACTCTCTGCGTCAGATTC  A215L-SENSE / 7726
GAATCTGACGCAGAGAGTTCTATGCGC  A215L-ANTI / 7727
CTGATTACACCCAAGGGGGATG       E354K-SENSE / 7792
CATCCCCCTTGGGTGTAATCAG       E354K-ANTI / 7793
cccttccgcatagannngcctgcgtcagt        T214N-Sense / 8202
actgacgcaggcNNNtctatgcggaaggg        T214N-Anti / 82033
```

```
GCAATCAAATCGCTCCGGATACTGC    I232A-SENSE / 6911
GCAGTATCCGGAGCGATTTGATTGC    I232A-ANTI / 6912
```

```
CCATTCCATCAAGGTTTTGG    H245Q-SENSE / 9128

CCAAAACCTTGATGGAATGG    H245Q-ANTI / 9129
```